



Charge Density Quantification and Antimicrobial Efficacy

by Nicole Zander, Julia Leadore, and Joshua A. Orlicki

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Nicole Zander
Dynamic Science, Inc.

Julia Leadore
Oak Ridge Institute for Science and Education

Joshua A. Orlicki
Weapons and Materials Research Directorate, ARL

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14. ABSTRACT Emerging threats to soldiers on the battlefield include traditional dangers such as conventional weapons and chemical or biological warfare agents. A less obvious threat is represented by the growing numbers of serious bacterial and fungal infections. Reducing overall warfighter susceptibility to opportunistic infections would improve force readiness in all operational environments. The capability of a material to autonomously decontaminate <i>in situ</i> with an active additive is therefore highly desirable and may increase the warfighter's safety and reduce the logistical burdens associated with decontamination operations. However, to maintain the critical performance characteristics of the coating or fabric, a minimal amount of active material is preferred, reducing the overall impact on bulk physical properties. Permanent, nonleaching antimicrobial surfaces were prepared by covalent attachment of polyelectrolytes to silane modified glass slides. The efficacy and mechanism of biological decontamination depend on the charge density, the length of the hydrophobic chain in the quaternary ammonium groups, and the molecular weight of the cationic polymer. Structure-activity relationships of linear polymers, as well as small molecules covalently attached to a glass substrate, were examined to determine structures that achieve maximum antimicrobial efficacy. Charge quantification was measured using UV-Vis spectroscopy with a sodium fluorescein complexation method.					
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1. Background

Today's warfighter faces not only the expected threats of enemy fire and roadside bombs but also the less obvious threats from bacterial and fungal infections. These infections can arise from the poor hygienic conditions inherent to the field or from opportunistic events such as wounds. Approximately 20% of combat wounds result in infections, which increase hospitalization time, morbidity, and mortality. In addition, the decontamination of vehicle interiors and exteriors, buildings, and fabric surfaces following exposure to a biological contaminant presents many logistical problems. The capability of a material to autonomously decontaminate *in situ* with an active agent is therefore highly desirable and may reduce the logistical burdens associated with decontamination operations (1).

Although there are many candidates for self-decontaminating materials, permanent, nonleaching polycationic surfaces have recently attracted much attention. These materials consist of a variety of linear and dendritic quaternary ammonium and phosphonium polymeric salts. Many of the polymers are covalently attached to a substrate via alkyl silane coupling and do not leach out into surrounding mediums, preventing environmental endangerment or the need for coating reapplication.

Even though the precise method of bacterial cell death from these materials remains unknown, the charge density of the polymer plays an important role in disruption of the cellular membrane. Kügler et al. determined that there is a charge density threshold above which cell death is rapid (2). Determining the correlation between charge density and biocidal efficacy could thus be a useful tool to evaluate coating systems.

2. Materials and Methods

2.1 Materials

Aminopropyltrimethoxysilane (APS), propyltrimethoxysilane (PTMO), polyvinyl pyridine (PVP) (160,000 MW), 1,4-dibromobutane, sodium fluorescein, cetyltrimethyl-ammonium chloride, and all alkyl bromides and solvents were purchased from Sigma Aldrich. Glass microscope slides were purchased from VWR International, LLC. Solvents and reagents were used without further purification.

2.2 Methods

Two distinct methods were employed to fabricate permanent antimicrobial surfaces. The first method (method A) consisted of grafting polyvinyl pyridine chains to a glass substrate (3). The tertiary amines were then alkylated with various chain length alkyl bromines. In the second

method (method B), glass substrates were treated with amine terminated and methyl terminated silanes at various ratios. The amines were then exhaustively alkylated with methyl iodide to form quaternary ammonium salts.

2.2.1 Method A

Standard glass microscope slides were cut in half and flame cleaned. They were immersed in a 1 weight-percent APS solution in 90:10 ethanol:water for 1 min at room temperature. The slides were rinsed twice with ethanol and baked at 70 °C for 1 hr. They were then immersed into a mixture of 90-mL nitromethane, 9-mL 1,4-dibromobutane, and 0.12-mL triethylamine in a 30-mL glass beaker and heated at 60 °C for 2 hr to attach the bifunctional cross-linker (figure 1).

Next, they were rinsed with nitromethane and dried in a clean stream of nitrogen. One half gram of PVP was dissolved in 20-mL nitromethane at room temperature. The treated slide and 2.5 mL of an alkyl bromide were then added. The reaction was heated at 75 °C for 24 hr. The slides were then rinsed with acetone, then methanol and air dried.

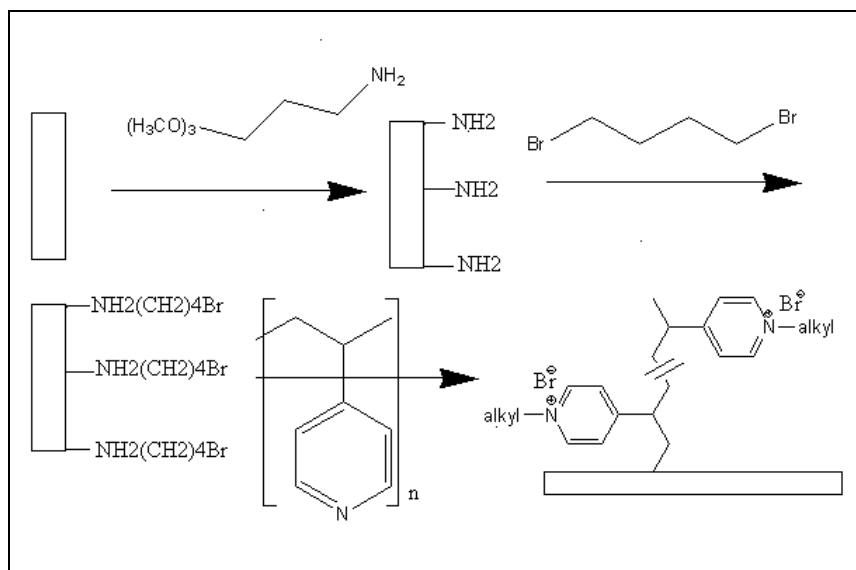


Figure 1. Method A sample preparation on glass substrate.

2.2.2 Method B

Standard glass microscope slides were cut in half or quarters and cleaned with either a TL1 wash or Piranha etch procedure. They were immersed in mixtures of 1 weight-percent APS and PTMO solutions in 90:10 ethanol:water from 0% to 100% of each component for 1 min at room temperature (see table 1). The pH of the PTMO solution was adjusted to 4.5 with glacial acetic acid. The slides were then dip rinsed in ethanol or deionized (DI) water, rinsed with DI water, dried with nitrogen, and baked at 70 °C for 1 hr. Next, they were then placed in 20 mL of methanol and 50 μ L of methyl iodide and heated for 72 hr at 70 °C in a shaking heater block.

Table 1. Method B alkyl silane treatment solution compositions.

Sample	Volume-Percent PTMO	Volume-Percent APS
A	100	0
B	80	20
C	60	40
D	40	60
E	20	80
F	0	100

2.3 Surface Analysis

The surfaces of these materials were examined by ultraviolet-visible spectroscopy (UV-VIS). The slides were soaked in a 1 weight-percent sodium fluorescein aqueous solution for 5 min, rinsed thoroughly with DI water, and placed in a 0.1 weight-percent aqueous cetyltrimethylammonium chloride solution. The solutions were shaken for 10 min on a wrist shaker; 10 volume-percent of PBS buffer (pH = 8) was then added. The solution was analyzed on a Perkin-Elmer Lambda 950 UV-VIS spectrometer in transmission mode. The scan range was 400–600 nm, with the maximum absorbance at 501 nm. The molar extinction coefficient was independently calculated to be $77 \text{ mM}^{-1}\text{cm}^{-1}$. Goniometry was used to measure the water contact angle with video microscope images and a Labview automated fitting program.

X-ray photoelectron spectroscopy (XPS) data was obtained using a Kratos Axis 165 XPS system equipped with a hemispherical analyzer. A 100-W monochromatic $\text{Al K}\alpha$ (1486.7 eV) beam irradiated a 0.3×0.7 -mm sampling area. Survey scans were taken at pass energy = 80 eV. Elemental high resolution scans for C 1s, O 1s, N 1s, and F 1s were taken at pass energy = 20 eV. CASA XPS software was utilized for all data analysis.

2.4 Antimicrobial Analysis

The antimicrobial efficacy of the surfaces was examined against *E. coli* and *S. aureus* using a standard test method for nonleaching hydrophobic materials (ASTM E 2180) (4). Briefly, 1×10^6 cells in agar slurry was loaded onto the test surface and incubated at 37 °C for 24 hr. The inoculum was recovered in a neutralizing medium of Dey Engley Broth via sonication and vortexing. The broth was diluted with 0.85% saline water and plated. Colonies were counted after 48 hr, and treated samples were compared to controls consisting of either APS glass or PVP glass.

2.5 Assumptions and Calculations

Although the exact mechanism of antimicrobial action remains unknown, researchers have determined that electrostatic interactions play an important role in the adsorption of the biocide onto the cell wall (2). One accepted theory is that the negatively charged cell walls of bacteria are attracted to the positively charged surfaces created by quaternary ammonium salts. The bacteria's natural counter-ions are then released, and the cell wall is destabilized due to electrostatic repulsion (3). The effects of surface charge alone may initiate cell death, but other

parameters such as length of the hydrophobic chain on the amine could also be important. K gler et al. determined that a charge density threshold exists, above which bacterial death is rapid (2). In fact, they found that the typical charge of a bacterial outer membrane is $\sim 10^{13}$ N⁺/cm. Thus, an equal or greater charge on the substrate surface is needed to drive the release of the bacterial counter-ions and initiate cell death.

The purpose of this study was to determine the correlation between charge density and antimicrobial efficacy and compare the surface analysis techniques of UV-VIS and XPS for quantification of surface quaternary ammonium salts. In the former charge quantification method, a divalent salt of the dye sodium fluorescein binds only to quaternary ammonium salts. After thoroughly washing the surface to remove excess dye, the bound dye molecules are disassociated from the surface by treatment with a detergent solution. The resulting dye concentration of the liquid is proportional to the amount of dye bound to the surface, and the charge density can be calculated according to Beer's Law, adapted using equations 1–9. The charges measured correspond to quaternary ammonium groups forming an ionic complex with the dye.

$$\text{Abs}_{501} = \epsilon_{501}[\text{Dye}]c. \quad (1)$$

Once [Dye] is determined, the total quantity of dye attached to the surface is calculated and used to determine the charge density.

$$(A) \text{ molecules of charge} = (M * V * 6.023 \times 10^{23}) * 2, \quad (2)$$

and

$$(B) \text{ charge density} = B/\text{area}, \quad (3)$$

where

$$\text{Abs} = \text{absorbance of sodium fluorescein at } \lambda_{\text{max}} = 501 \text{ nm}, \quad (4)$$

$$\epsilon = \text{molar extinction coefficient} = 77 \text{ mM}^{-1}\text{cm}^{-1}, \quad (5)$$

$$c = \text{path length, } 1.0 \text{ cm}, \quad (6)$$

$$M = \text{molarity}, \quad (7)$$

$$V = \text{dilution volume} = 0.027 \text{ L}, \quad (8)$$

and

$$\text{area} = \text{surface area of substrate, } = 18.75 \text{ cm}^2. \quad (9)$$

XPS provides chemical bonding as well as quantitative elemental information. The atomic percentage of amine groups, as well as the surface composition of quaternary ammonium ions, can be determined. Due to the complexity of method A samples, only method B samples were analyzed by XPS.

3. Results and Discussion

3.1 Method A Results

Polyvinylpyridine was alkylated with a variety of different chain lengths from 4 to 10 carbons. The UV-VIS results are shown in table 2, and antimicrobial data is displayed in figure 2. Figure 3 displays the contact angle data for the series, showing increasing hydrophobicity with increasing alkyl chain length. The four-carbon chain achieved the highest absorbance and biocidal efficacy. Compared to the other samples, the PVP with six-carbon hydrophobe also had a fairly high absorbance and biocidal efficacy. There appears to be a critical chain length of less than eight carbons for achieving good bacterial kill. In general, shorter alkyl chains are less effective at puncturing a cell wall but may allow higher alkylation and hence quaternization of the PVP tertiary amines due to less steric hindrance. Chains longer than 10 carbons stick together due to hydrophobic interactions (3). The absorbance of the PVP-C6 was less than 1/6 of the PVP-C4, but both materials achieved similar antimicrobial efficacy. A simple explanation could be that the longer six-carbon chain limited the extent of alkylation compared to the four-carbon chain. Thus, the absorbance for the former was reduced. But the charge density of the PVP-C6 was above the critical threshold value nonetheless and yielded similar bacterial kill. Tiller et. al. also examined a lower molecular weight PVP (60,000 KDa) but found it was much less effective in achieving bacterial kill due to a lower charge density (3).

Table 2. UV-VIS data for PVP quaternary ammonium salts.

Material	Absorbance $\lambda = 501 \text{ nm}$	Charge Density
PVP-C4	3.356	7.6×10^{16}
PVP-C6	0.501	1.1×10^{16}
PVP-C7	0.181	4.1×10^{15}
PVP-C9	0.125	2.8×10^{15}
PVP-C10	0.173	3.9×10^{15}
Control	0.00913	2.1×10^{14}

3.2 Method B Results – UV-VIS

Due to the complexity of the grafted charged polymer system, a model system of small molecule amine and methyl terminated silanes was used to study charge density. The amine terminated silanes contained the reactive groups that could undergo further chemistry via methylation by methyl iodide, whereas, the methyl terminated silanes served as unreactive surface blocking molecules. In theory, a single monolayer of silanes was deposited on the substrate, with varied amine surface coverage. Exhaustive alkylation of the amines with methyl iodide formed quaternary ammonium salts on the surface. UV-VIS, XPS, and contact angle were examined

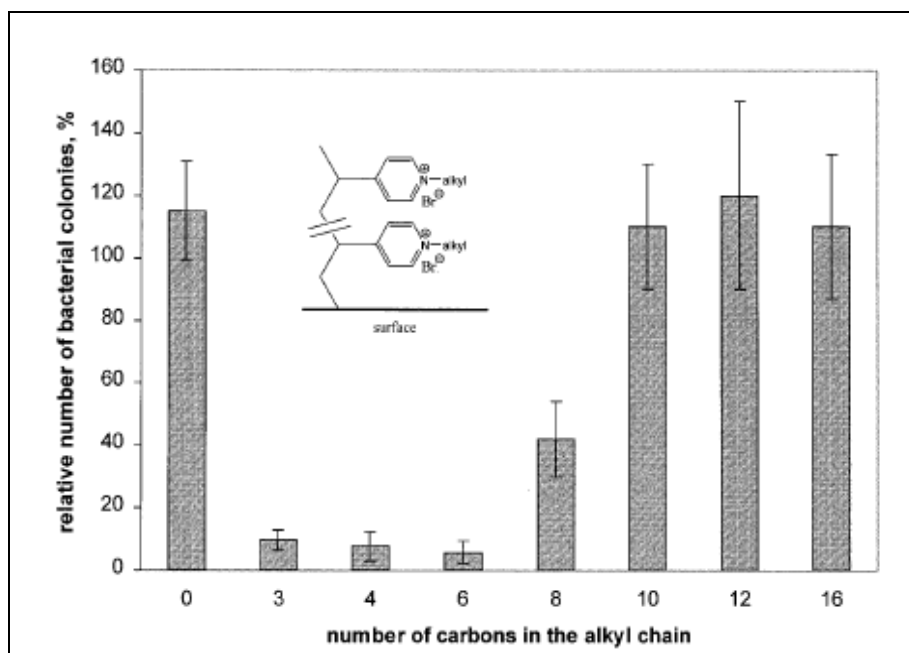


Figure 2. Reduction of *S. aureus* as a function of alkyl chain length (3).

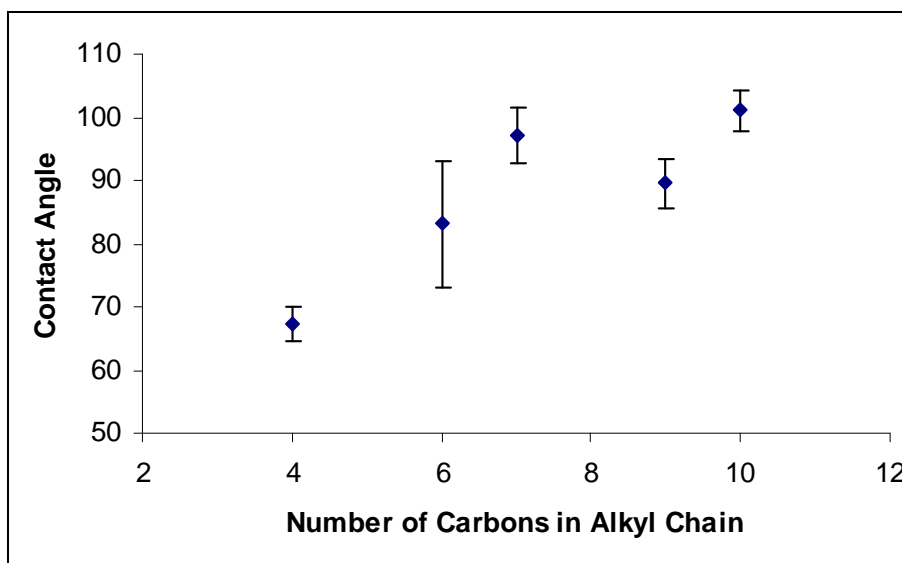


Figure 3. Contact angle of PVP grafted films.

to probe the surface chemistry of the samples. Correlation of charge density and antimicrobial efficacy was unsuccessful because none of these samples showed significant bacterial reduction. Much shorter total chain lengths and lower charge densities as compared to method A samples are most likely contributing factors (as will be discussed next). In addition, the one-carbon hydrophobes on the quaternary ammonium salts were mostly likely too short to puncture cell walls (5). Table 3 shows the charge density of controls and samples. Absorbances and hence charge densities are, in general, one to two orders of magnitude lower for these samples

Table 3. UV-VIS data of varied aminosilane model system.

APS (%)	PTMO (%)	Absorbance (Control) $\lambda = 501 \text{ nm}$	Absorbance (Quat) $\lambda = 501 \text{ nm}$	Charge Density (Control)	Charge Density (Quat)
0	100	0.002067	0.016087	4.7×10^{13}	3.6×10^{14}
20	80	0.007733	0.005561	1.7×10^{14}	1.3×10^{14}
40	60	0.025442	0.087748	5.7×10^{14}	2.0×10^{15}
60	40	0.048315	0.027554	1.1×10^{15}	6.2×10^{14}
80	20	0.048427	0.138875	1.1×10^{15}	3.1×10^{15}
100	0	0.166408	0.407844	3.7×10^{15}	9.2×10^{15}

when compared to the PVP quats. This is not unexpected because the grafted polymers have ~1500 monomer units per chain and substantially more sites for creating charged species. For the PVP and APS samples, the controls have a charge density ranging from 1×10^{13} to 9.4×10^{14} . The glass slide is probably absorbing some of the dye molecules and could vary based on imperfections on the surface. In addition, there appears to be a trend of increasing absorbance with increasing amine on the control samples (see figure 3). The pH of the deionized water was tested to be 5.6. A buffer of pH = 8 was added before UV-VIS measurements were taken to deprotonate the nonquaternized amines. The actual pH of this solution was measured to be about 6.8–7.2. Thus, it is possible that some of the free amines on the slide are being protonated by the water. The trend follows expectations of a higher absorbance for samples with increased amine coverage.

3.3 Method B Results – XPS

Figure 4 displays XPS results for the control and quaternary ammonium salt samples. There appears to be a trend of increasing nitrogen: carbon (N/C) with increasing APS fraction of treatment solution. The N/C ratio is lower at all sampling levels for the quaternary ammonium salts due to the three additional carbons added per nitrogen from the quaternization reaction. Table 4 displays the XPS elemental data, with increasing N at higher APS fractions as expected. The data for the quaternary samples is shown in table 5, with a representative survey spectrum shown in figure 5. A stoichiometry of 1:1 of nitrogen:iodine (N:I) was expected if all primary nitrogens were fully alkylated with methyl iodide to form charged nitrogens with iodide as the counter-ion. The alkylation seems to vary randomly as the 100% APS sample is nearly fully alkylated with an N:I ratio of 4.2:3.4. However, the 20% APS sample is only partially alkylated. This indicates that it is not necessarily a steric hindrance issue that is inhibiting full quaternization. Other factors such as reaction time, temperature, solvent, or additional experimental conditions could be preventing exhaustive alkylation. Table 6 displays data from an experiment to determine if large variations in the surface chemistry could be detected with orders of magnitude variation in APS treatment fractions. As can be seen, at any APS treatment fraction (0.1% and above), the amine surface atomic percent remains more or less the same.

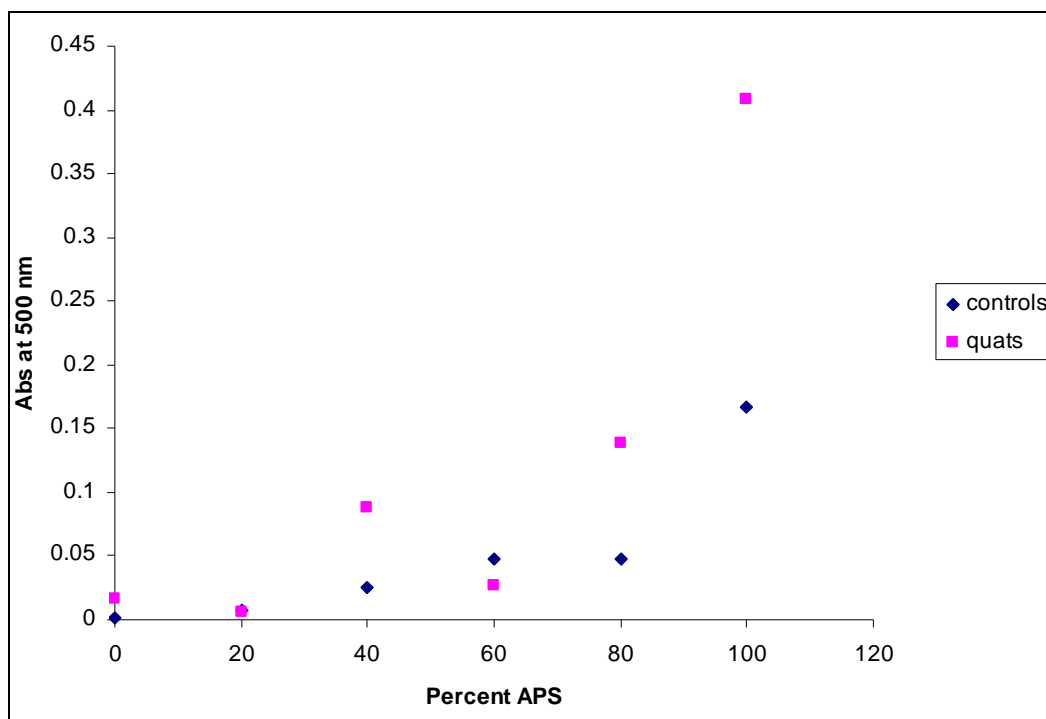


Figure 4. Absorbance vs. percentage of surface coverage of amine groups.

Table 4. XPS data of APS/PTMO control samples.

Description	O	C	N	Si
APS	28.6488	45.71	6.613	19.03
80% APS	26.9	45.38	6.22	21.49
60% APS	27.75	44.79	7.7	19.76
40% APS	43.44	28.71	3.857	23.99
20% APS	34.94	38.62	2.792	23.64
PTMO	42.54	29.16	1.107	27.2

Table 5. XPS data of APS/PTMO quaternized samples.

Description	O	C	N	Si	I
APS-CH3I Q	21.07	50.92	4.177	20.47	3.356
80% APS-CH3I Q	21.62	47.33	0	26.85	4.204
60% APS-CH3I Q	21.46	52.35	4.184	20.41	1.594
40% APS-CH3I Q	24.38	50.81	0.79	23.06	0.9637
20% APS-CH3I Q	23.73	53.63	1.689	20.68	0.2664
PTMO-CH3I Q	50.81	21.53	0.1583	27.5	0

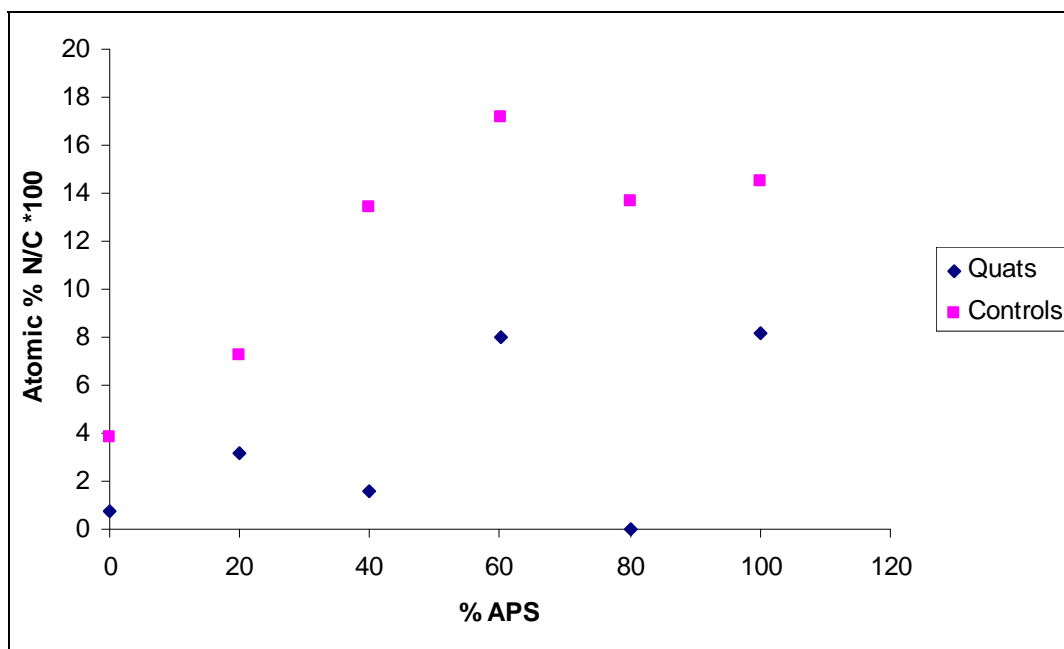


Figure 5. XPS N/C ratios of method B controls and quaternary nitrogen samples.

Table 6. XPS data of APS/PTMO control samples.

APS (%)	C	O	N	Si
0	19.84	51.66	0.8411	27.66
0.1	17.8	51.72	1.838	28.59
1	21.29	49.47	1.899	27.34
10	25.04	46.81	1.322	26.83
100	22.985	46.72	2.003	27.93

Thus, increasing the volume fraction APS (relative to PTMO) does not appear to have any effect on increasing the rate of APS reaction with the substrate. Other experiments with 100% volume fraction APS yielded 2–3× higher amine surface coverage. Previous researchers have determined that 1–2 atomic-percent nitrogen constitutes a single monolayer of coverage (6). Hence, in some sample preparations, two or three monolayers were formed on the surface.

3.4 Discussion

Previous research had indicated that surface amines could be tuned by controlling the volume fraction of amino and methyl terminated silanes (7). Although the general trend of increasing absorbance or sodium fluorescein, amine content or N/C ratio with increasing APS fraction was observed, it was weaker than expected and not reliably reproducible. Several contributing factors such as silane reaction kinetics, solid phase chemistry reactions, and instrument sensitivity limits were probably to blame. For example, it was assumed that the PTMO and APS

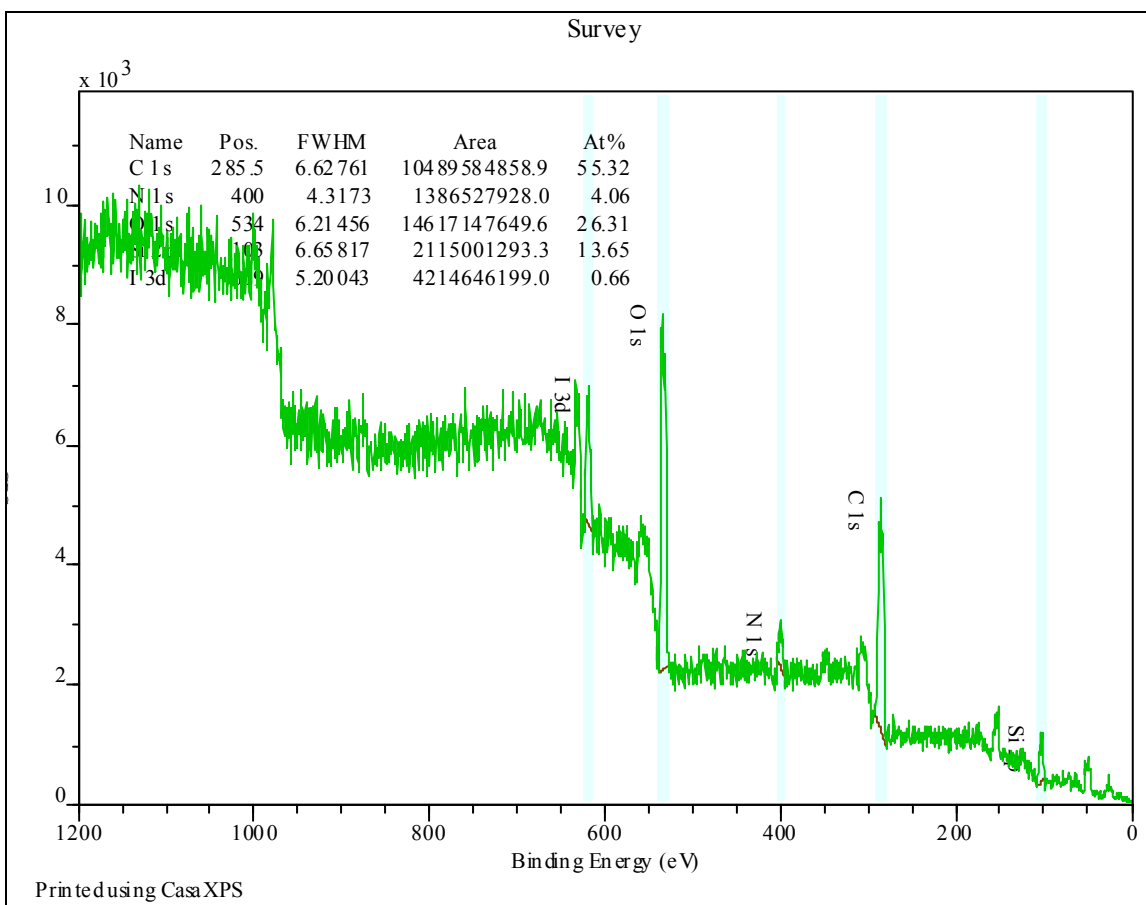


Figure 6. Survey spectrum of quaternary ammonium salt surface.

silane solutions would react equally fast with the etched glass substrate and not inhibit one another. Although further studies would be required to confirm this notion, varied reaction kinetics and/or inhibition could partially explain some of the observed results. Also, as just mentioned, APS formed varied monolayer coverage during independent sample preparations. APS has been known to form networks. Some researchers prefer to use dry toluene to prevent water from catalyzing the network formation reaction. Using dry solvents in this study may have been difficult because the hydrolysis of the methoxy groups in PTMO requires an acid catalyst. Furthermore, the mixing of the basic APS solution and the acidic PTMO solution could have, in effect, neutralized the resulting solution and limited hydrolysis of either component. In addition, the surface charge density technique was developed for grafted polymer chains, each of which contain over a hundred dye-binding sites as opposed to one binding site per two surface amines. Thus, a much higher instrument sensitivity is required to distinguish samples at this 100-fold reduced range.

4. Conclusions

The surface charge density is an important contributing factor to the antimicrobial efficacy of coating (2, 8, 9). UV-VIS spectroscopy offers a quick and inexpensive means to quantify the surface charge of a quaternary ammonium salt surface coating (8). A good correlation between charge density and antimicrobial efficacy was observed for grafted polymer chains prepared in method A. No microbial reduction was observed with the method B model system. Thus, a charge and kill correlation could not be determined. An attempt to relate volume fraction of amine terminated silanes, charge density, and XPS amine surface coverage was performed. A weak correlation of increasing amine surface coverage and charge density was observed for samples treated with a higher volume fraction of amine terminated silane. Strong correlations could not be seen due to network formation of APS molecules, reaction kinetics of the PTMO and APS silanes, greatly attenuated amine sites for surface charge generation, and incomplete and random methylation of surface amines.

5. References

1. Rawlett, A.; Orlicki, J.; La Scala, J.; Piehler, L.; Zander, N.; Smith, P.; Demaree, D.; Kosik, W.; McKnight, S.; Rice, N.; Kagumba, L.; Giaya, A. *Nanoengineered Additives for Active Coatings*; ARL-TN-273; U.S. Army Research Laboratory: Aberdeen Proving Ground, MD, April 2007.
2. Kügler, R.; Bouloussa, O.; Rondelez, F. *Microbiology* **2005**, *151*, 1341–1348.
3. Tiller, J.; Liao, C.; Lewis, K.; Klivanov, A. *Proceedings of the National Academy of Sciences* **2001**, *98*, 5981–5985.
4. ASTM E 2180. Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials. *Annu. Book ASTM Stand.* **2007**.
5. Chen, C.; Cooper, S. *Biomaterials* **2003**, *23*, 3359–3368.
6. Kurth, D.; Bein, T. *Langmuir* **1993**, *9*, 2965–2973.
7. Robinette, J.; Bujanda, A.; Jensen, R.; McKnight, S. *AIChE J.* **2006**.
8. Chen, C.; Beck-Tan, N.; Dhurjati, P.; van Dyk, T.; LaRossa, R.; Cooper, S. *Biomacromolecules* **2000**, *3*, 473–480.
9. Lee, S.; Koepsel, L. S.; Morley, R.; Matyjaszewski, S.; Sun, K. Y.; Russell, A. *Biomacromolecules* **2004**, *3*, 877–882.

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